0031-6997/85/3704-0365$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 37, No. 4
Printed in U.S.A.

Neurobehavioral Toxicology of Trialkyltins

D. E. MCMILLAN and G. R. WENGER

Department of Pharmacology and Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas

I. Introduction 365
II. Triethyltin 365
A. Behavioral effects 365
1. Unconditioned behaviors 366
2. Schedule-controlled behavior 367
B. Effects on the nervous system 367
1. TET and edema in the central nervous system 368
2. TET and neurotransmitters 368
3. Summary of TET effects 368
III. Trimethyltin 368
A. Behavioral effects 368
1. Unconditioned behavior 370
2. Conditioned behavior 372
B. Tissue distribution of TMT 372
C. Effects of TMT on the nervous system 372
1. TMT and nerve damage 372
2. TMT and neurotransmitters 374
D. Summary of TMT effects 374
IV. Neurobehavioral toxicology of alkyltins: problems and promises 375

I. Introduction

ALKYLTIN compounds have been used widely as stabilizers in the plastics industry (87, 95); as miticides, fungicides, bactericides, algicides, insecticides, and chemosterilants (47, 99); for a variety of agricultural applications; as a preservative in wood products and the textile industry (99); in hospitals as disinfectants (45); and for a variety of other industrial applications (76). It has been estimated that at one time more than 25,000 metric tons of organotin compounds were used annually, mostly as antifouling compounds for ships' hulls and for other marine structures (36).

Although the exposure of animals, including man, to organotins has only recently begun to receive major attention, there have been several well-recognized poisoning incidents. Perhaps the most dramatic of these episodes occurred in France in the 1950s. Inorganic tin compounds were being used to treat skin disorders. One of the commercial preparations marketed for this purpose was contaminated with alkyltins, particularly triethyltin (TET), and this resulted in more than 100 deaths when it was given p.o. (5). More recently, two reports of chemists or chemical workers exposed to alkyltins, primarily trimethyltin (TMT), have emphasized the occurrence of central nervous system (CNS) symptoms including confusion, disorientation, insomnia, memory loss, mood changes, anorexia, and general malaise (38, 82). These poisonings resulted from acute exposure to large doses of alkyltins. Almost nothing is known about the effects of chronic low level exposure to alkyltins.

Although the toxicity of alkyltins has been recognized for 100 yr (104), much of the recent interest in these compounds comes not from the standpoint of environmental toxicology, but rather from the possibility that alkyltins might become important research tools for the study of brain function. Early studies with TET showed that it produced a progressive encephalopathy characterized by severe brain edema (64), suggesting that TET exposure might be used as a model for some types of degenerative disorders (40). The report by Brown et al. (11) that TMT produced bilateral symmetrical neuronal damage in hippocampal areas has led to an explosion of interest in this compound.

II. Triethyltin

A. Behavioral Effects

1. Unconditioned behaviors. Acute administration of TET to mice (40) or rats (67, 79) results in a decreased spontaneous motor activity. With large doses (8–10 mg/kg), mice are affected within 10–30 min. About 1 h after administration, the mice begin to show signs of recovery,

and they appear to have recovered completely from acute effects within a few hours. Although many effects of TET in rats appear to be reversible in those animals that survive (88), when the effects of acute doses of TET are studied on the total motor activity of the rat over a 24-h period, motor activity in a running wheel remains decreased for as long as 10 days (67).

Several investigators have studied the effects of acute administration of TET to neonatal animals and then followed changes in motor activity over time (42, 78). In rats given TET on day 5 postpartum, activity was increased by about 20% for about the first 60 days (42), while at 90 days small decreases in activity were observed. However, Reiter et al. (78) found small (10–20%) but persistent activity increases in adult rats tested in a figure-eight maze after treatment with 3 or 6 mg of TET per kg on day 5 postpartum, and McMillan et al. (67) found that the administration of 3 mg of TET per kg to adult rats produced small increases in motor activity that became apparent approximately 2 wk after administration. It seems clear that increased motor activity can be observed after acute administration of TET and that these activity increases can be persistent, but the degree to which such activity increases depend on the age of the rat when exposed to TET and whether or not the effects are reversible are still unclear.

Reiter et al. (79) studied the effects of chronic exposure to TET in rats exposed to TET in the drinking water (5, 10, and 20 ppm) for a period of 4 wk, which resulted in average daily doses of 0.40, 0.66, and 0.82 mg/kg/day for the three increasing concentrations of TET. After 2 wk of exposure to 10 ppm in the drinking water, activity was decreased by about 50%, but these effects were reversible within a month. Rats with TET in the drinking water showed decreased fluid intake and showed weight gain, but this did not account for the changes in activity, since the addition of quinine to the drinking water of rats produced similar decreases in water intake without decreasing motor activity. Suzuki (89) also noted less activity in adult rats with chronic exposure to 5 ppm of TET in the drinking water, but these were only qualitative impressions, and the total daily TET intake could not be determined. There have not been long-term studies of rats chronically exposed to low doses of TET in the drinking water to determine if any hyperactivity similar to that seen with acute TET administration develops after the discontinuation of chronic TET administration and continues throughout the life of the rat.

It has been well recognized that the administration of TET produces a weakness and eventually a spastic paresis and paraplegia of the hindlimbs (6). These effects may contribute to some of the motor activity decreases seen in the early stages of TET poisoning. In addition to effects on the central nervous system, this hindlimb paralysis appears to be due to direct effects of TET on the neuromuscular junction and perhaps on the muscle as well. TET decreases release of acetylcholine at the neuromuscular junction (6, 7, 69), but it probably also affects the membrane potential of the muscle fibers directly (7). However, the development of hindlimb paralysis also correlates with the development of morphological changes in the spinal cord (86), suggesting that effects on the central nervous system also contribute to the paralysis.

2. *Schedule-controlled behavior.* There have been a number of studies during the past few years showing effects of TET on schedule-controlled behavior in several species. Rastogi et al. (77) studied the effects of TET on rats responding under a multiple, fixed-ratio, fixed-interval schedule (mult FR FI) of food presentation. Under this schedule, in the presence of one stimulus condition, animals are required to emit a fixed number of responses within a specified time period in order to obtain a food pellet (FR). In the presence of a different stimulus, the first response that occurs after a fixed time period has elapsed produces the food pellet (FI). This schedule has been used to study the effects of a large number of drugs, because the FR component generates a steady rapid rate of responding, while the rate of responding during the FI component shows a gradual increase throughout the FI duration. A dose of 1.0 mg/kg was without clear effect on responding under either schedule component; however, 3.0 mg/kg decreased rates of responding to almost zero. Within 24 h, rates of responding had returned to control levels. A 5.6-mg/kg dose was lethal. Wenger and McMillan (100) demonstrated the effect of TET on the responding of BALB/c mice responding under a mult FR 30-response FI 600-s schedule of milk presentation. At 3 h after doses of 5, 7.5, or 10 mg of TET per kg, responding under both schedule components was markedly depressed. This effect was of relatively short duration, and the time of recovery was dose dependent with slower recovery after higher doses. At the highest dose, 10 mg/kg, responding had partially recovered 27 h after administration, and by 51 h responding was back to control levels. In mice exposed to repeated administration of 7.5 mg of TET per kg at 2-wk intervals, no evidence of cumulative or diminished effects was observed. Pigeons are more sensitive to the effects of TET on schedule-controlled behavior than are rats or mice, with rate-decreasing effects on responding under a mult FR FI schedule in the birds being quite marked 3 h after i.m. administration of 1.0 mg/kg (66). With higher doses, recovery is slower in pigeons, but as with rats (77), recovery occurs within a few days.

In experiments using other complex schedules of reinforcement, the effects of TET have been quite consistent. Wenger et al. (103) found that doses of TET above 3.0 mg/kg reduced the rate of responding and disrupted the temporal pattern of responding in rats under a schedule that reinforced only responses separated from each other by 10–14 s. In those animals that survived the

experiment, disruption of the temporal pattern of responding was correlated with the general health of the animal, and all surviving animals eventually showed complete recovery of the pattern of responding. Idemudia et al. (48, 51) studied the effects of TET on matching-to-sample behavior (the subjects were required to choose between two stimuli, one of which was identical to a stimulus present before a short delay period) in the pigeon. Although changes in matching accuracy were observed, marked decreases in rate of responding occurred at the same doses. All animals showed a recovery within a few days (depending on dose) for both rate of responding and matching accuracy. Administration of doses of 1.75–5.6 mg of TET per kg at 2- to 3-wk intervals did not appear to produce cumulative effects in these birds (49).

Other investigators also have studied the effects of chronic TET administration on schedule-controlled behavior. Dehaven et al. (27) administered doses of 0.5, 1.0, and 1.5 mg of TET per kg twice weekly for 6–8 wk. Rates of responding under the FR 30 schedule were reduced by about 50% after the highest dose (1.5 mg/kg), but the two lower doses were without clear effects. Under a FI 1-min schedule, the lower rates of responding were decreased by about 50% by a 1.0-mg/kg dose, suggesting that behavior maintained under the FI 1-min schedule was more sensitive to TET than that maintained under the FR-30 schedule. Animals under both schedules were maintained at 80% of their free-feeding weights. Other rats maintained at higher body weights and trained to respond under the FI 1-min schedule were affected at the lowest dose (0.5 mg/kg), suggesting that the effects of TET depend on the dose, the schedule of reinforcement maintaining the behavior, and the degree of food deprivation of the animal. It is particularly interesting to note that, in some of these experiments, the fourth of the biweekly TET administrations produced smaller decreases in response rate than did the first injection, which is indicative of tolerance development. The observation, that the rats maintained at higher body weights were more affected by TET, is an extension of earlier data on feeding behavior developed by these same investigators (26).

Although most of the studies indicate that the acute effects of TET on schedule-controlled behavior are reversible in surviving adult animals, as is the case with motor activity, the generalization may not hold when animals are given TET during the postnatal stage of development. Harry and Tilson (43) gave rats 3.0-mg/kg doses of TET on day 5 postnatally and then tested the animals as adults under a variable-interval 15-s schedule of food presentation (responses were reinforced after variable time intervals which averaged 15 s). Female rats tested at 90 days of age responded at about 80% of the rate of responding of control rats under this schedule of reinforcement, while male rats exposed to TET as neonates responded at about 140% of the rate of responding of control rats under the same schedule. Such data suggest long-term effects of TET on behavior as well as raising the issue of sex differences in response to TET.

B. Effects on the Nervous System

1. TET and edema in the central nervous system. Many investigators have replicated the finding of McGee et al. (64) that TET produces a severe edema in the central nervous system. Following TET administration, the total water content of the brain increases (4). Fluid appears to accumulate between the myelin layers, which results in a splitting of these layers (2, 57, 59). Although both grey and white matter are affected, white matter is more severely affected (2, 56). While the edema is pronounced in the central nervous system, little edema appears to occur peripherally (53, 85).

TET inhibits a number of metabolic activities in the brain. In in vitro studies on respiration, a concentration of 1×10^{-5} M reduced respiration by 60% (10). Oxidative phosphorylation is inhibited (10, 41), but the relationship of inhibition of oxidative phosphorylation to the development of edema remains unclear (37). Glutamate oxidation, and to a lesser extent succinate oxidation, are also inhibited (10), as is glucose oxidation in brain slices. At higher concentrations, pyruvate inhibition occurs (58).

Although a variety of such metabolic changes occur in the brain, it is difficult to relate them to the edema and subsequent vacuolization (37, 41). It is possible that a direct effect not based on metabolic changes is responsible for the pathology. A number of investigators have pointed out that the accumulation of water in myelin is accompanied by an accumulation of sodium ion (37, 55, 56), and perhaps at least the initial stages of myelin swelling result from an increased ion transport into myelin followed by an obligatory movement of water (53). Consistent with this interpretation is the fact that replacement of the mobile ions with isotonic sucrose prevents myelin swelling in vitro (53). It is not clear how TET produces changes in ion permeability. Lock (59) identified a high affinity binding site for TET in rat myelin, but unfortunately some agents that also appear to bind to this site do not produce edema, while other agents that do produce a similar edema in the CNS do not prevent TET binding. If this high affinity binding site is involved in the production of edema, it is clearly not the only factor.

Nagara et al. (73, 74) have observed that TET does not produce edema in the spinal cord of the quaking mouse. Although it is possible that the penetration of TET into myelin might be restricted by intralamellar tight junctions in this species, a metabolic abnormality that protects against TET is also possible (73, 74). Whatever the mechanism involved in the protection against TET in this strain, the existence of the protection emphasizes that genetic control occurs. Of particular inter-

est in these studies is that quaking mice showed the same lethargy after TET administration as did control mice; in fact, the quaking mice were slower to recovery from TET than controls. The lack of edema in the mice, coupled with an enhanced behavioral effect of TET, suggests that even when behavioral effects are observed in animals where edema occurs, the behavioral effects may not be a consequence of the edema.

The idea that the edema produced by TET is not necessarily responsible for all of the behavioral effects of TET receives additional support from a comparison of the time course of the morphological changes and the behavioral changes. Vacuoles appear in the brain of rats in about 3 h (55), whereas gross behavioral changes occur within a few minutes of TET administration. The production of flavor aversions in rats (54, 60) when TET is given shortly after consumption of a novel flavor suggests that aversive properties of TET are also produced rapidly after injection. Mice also show a rapid onset of effects after acute TET administration (39, 40). Although detailed studies of the onset of morphological change after TET administration have yet to be done, it appears that the occurrence of behavioral effects preceeds the development of clear morphological changes.

There is a similar divergence of behavioral and morphological effects during recovery from TET. For example, Graham et al. (41) gave high doses of TET in the drinking water and observed edema formation and an increased number of axonal neurofilaments and neurotubules, which paralleled a decrease in motor nerve conduction velocity during initial periods of intoxication; however, animals became asymptomatic and showed normal motor nerve conduction velocity 2 or 3 wk after discontinuation of TET, although the intramyelenic vacuoles and increased numbers of neurofilaments and neurotubules persisted. McMillan et al. (66) observed complete recovery from effects of acute TET administration on schedule-controlled behavior in the pigeon. Upon histological examination of the same birds used in the behavioral studies, continued myelin swelling was observed. Of course, it must be recognized that persistence of a lesion beyond the duration of a behavioral effect does not necessarily imply that the lesion was not originally involved in producing the behavioral effect. It has been recognized for many years that behavioral recovery is frequently possible despite the persistence of brain lesions.

2. TET and neurotransmitters. The effects of TET on various neurotransmitter systems in the brain may also relate to the behavioral effects of TET. TET depletes catecholamines in the brain. Moore and Brody (70) reported that whole brain norepinephrine is reduced 21 days after a single injection of 5 mg of TET per kg. This finding has been replicated by Robinson (81). More recently, Cook et al. (23) reported that dopamine depletion following TET lasts for about 7 days. TET also decreases whole brain levels of 5-hydroxytryptamine (29) and blocks convulsions produced by γ-aminobutyric acid (GABA) blockers (28).

In addition to its effects on neurotransmitter systems, TET inhibits forebrain growth (8), increases cerebrospinal fluid pressure (55), disrupts mitochondrial cristae (93), and reduces cerebral blood flow (62). The integrity of the blood-brain barrier appears to be maintained after TET (37).

3. Summary of TET effects. In summary, TET produces behavioral effects with a rapid onset after acute administration of high doses. In adult animals, these effects usually persist for only a few days, although there are a few reports suggesting that longer effects occur when sophisticated behavioral measures are used. In general, the effects of TET on behavior appear to be reversible, but effects occurring some months after TET have been reported when TET has been given to neonate rodents. The characteristic edema produced by TET comes on more slowly than the behavioral effects, although it is likely that at least some of the events leading to the production of the edema also promote changes in behavior. The edema proceeds to myelin splitting and eventually to the production of vacuoles. These morphological changes appear to outlast at least the acute behavioral effects. Despite the poor correlation between edema formation and behavioral change, it is difficult to imagine that the myelin swelling does not in some way contribute to the behavioral effects of TET. TET produces a host of metabolic changes in brain as well as interacting with various neurotransmitter systems. At this point in time, it is impossible to relate these complex effects to behavioral change.

III. Trimethyltin

A. Behavioral Effects

1. Unconditioned behavior. TMT has been shown to produce behavioral effects in a variety of species, including man. In most species, these effects are seen at doses lower than those required to produce behavioral effects with TET, but the rat seems to be an exception to this generalization. In spite of this difference between rats and other species studied thus far, the rat has been the species studied the most extensively, both from the standpoint of behavior and pathology. In the rat, TMT produces what has been called the "trimethyltin syndrome" (34). The syndrome in Long-Evans rats is characterized by spontaneous seizures, tail chasing, tail mutilation, and vocalization. Closely paralleling this cluster of behavioral effects are a tremor and hyperreactivity characterized by an increase in aggressive behavior which made the animals difficult to handle. Other workers have reported similar behavioral effects in other species following TMT administration. However, the degree of aggressive behavior may be somewhat species or strain dependent. In addition to studies on TMT in the Long-

Evans rat, aggression following TMT has been reported in one other species, the marmoset (1). In contrast, increases in aggression have not been reported in C57BL/6N or BALB/c mice (101), CFW or Grasshopper mice (46), albino (TEX:ICR) mice (24), Sprague-Dawley rats (103), and pigeons (50, 65, 66). The most consistent effect reported across all species and strains is a marked whole-body tremor.

The effect of TMT on motor activity has been determined in several species including the mouse, rat, and pigeon. In the C57BL and BALB/c mouse, doses of 1 and 3 mg/kg produced an initial 20–50% decrease in motor activity. In the C57BL strain, the decrease observed after 3 mg/kg was followed by a 100% increase in activity. The increase in activity gradually declined and returned to control levels by about 10 days after TMT exposure. An increase of smaller magnitude and shorter duration was observed in mice of the BALB/c strain (101). The decreases in activity were associated with changes in the normal circadian rhythm of activity, resulting in an increase in the percentage of total activity occurring in the light phase of the light:dark cycle. A single dose of TMT disrupted activity for 10–14 days in the two strains of mice.

Activity measurements in rats following TMT have been conducted most frequently in open field devices and figure-8 mazes, and few, if any, studies have monitored activity continuously following TMT administration. Ruppert et al. (84) exposed rats p.o. to 0, 5, 6, or 7 mg of TMT chloride per kg, and activity was assessed in a figure-8 maze for 1 h at 2 h, 4, 8, 16, and 32 days after administration of TMT. No significant behavioral effects were observed following doses of 0, 5, or 6 mg of TMT per kg. In rats exposed to 7 mg/kg, no effects were seen 2 h after exposure, but on subsequent tests, activity was increased by more than 100% above control values. The peak in the activity increase occurred at 16 days after TMT administration, with only a small diminution at 32 days. Activity was increased in the figure-8 portion of the maze without a corresponding change in the blind alleys of the maze.

Several other studies report the effect of TMT on activity of rats in an open field following exposure as adult animals. Johnson et al. (52) reported that, in rats exposed to TMT chloride and tested at three time periods (14–18, 56–60, and 106–110 days), a 5-mg/kg dose produced a 40% increase in activity only at the 14- to 18-day test period, while rats treated with 7 mg/kg showed similar activity increases at all three time periods. The same rats also had an increased water intake for the 3 wk following TMT administration. The peak in water intake of approximately 60% above control levels was observed 1 day after 7 mg/kg and 5 days after 5 mg/kg. Swartzwelder et al. (92) administered 7 mg of TMT per kg p.o. to adult male Long-Evans rats. Five wk after exposure, they were tested in an open field activity chamber. Compared to control rats, treated rats had a 3-fold greater level of activity in the open field. In addition, treated rats showed an altered response to *d*-amphetamine sulfate in the open field. In control rats, a dose of 0.5 mg of *d*-amphetamine per kg was without effect, while doses of 2 and 4 mg/kg increased activity. In contrast, *d*-amphetamine administered to TMT-treated rats in doses of 0.5 and 2 mg/kg increased rates of responding, and 4 mg of *d*-amphetamine per kg decreased activity in the open field. Swartzwelder et al. (90) reported more than a 3-fold increase over controls in the open field activity of Long-Evans rats administered 7 mg of TMT chloride per kg and tested in an open field 40 days after exposure. TMT also increased rat locomotor activity as measured by activity in a running wheel (67). Beginning 3 h after 3, 6, or 9 mg of TMT per kg, rats showed an approximate 50% decrease in running in a wheel, but on the next day, running had returned to control values in the rats receiving 3 mg of TMT per kg. Rats receiving 6 and 9 mg/kg showed significant increases in activity after the initial decrease. After 9.0 mg of TMT per kg, activity increases peaked 3 days after exposure at a value 3 times the control rate. Subsequently, running began to decline, and most rats died within a few days. After the 6.0-mg/kg dose, increased running (3–5 times the control value) was sustained for at least 2 wk.

In a similar study on the home-cage behavior of rats exposed to 0, 3, 5, or 7 mg of TMT per kg, Bushnell and Evans (13) reported that food consumption was reduced to 25% of control level 5 days after 7 mg/kg, and to 50% of control 2–3 days after 5 mg/kg. Water intake doubled within 2 days after 7 mg/kg and remained elevated for 14 days. Diurnal patterns of drinking and rearing were disrupted at all doses of TMT tested.

In a study on spontaneous motor activity in pigeons, Idemudia and McMillan (48) showed that i.m. doses of 1, 1.3, and 1.75 mg of TMT chloride per kg significantly disrupted activity in an Automex activity device. The two lower doses produced both increases and decreases in activity, while the highest dose, 1.75 mg/kg, produced only decreases in activity.

There are several reports on the effect of TMT on sensory function in laboratory animals. Walsh et al. (97) studied the effects of TMT on antinociception in Fischer 344 rats. Using a hot plate set at 57.5°C, no significant effects were observed with a TMT dose of 1.75 mg/kg. At a dose of 3.5 mg/kg, no effects were observed at 1, 7, or 14 days after administration. At 21 and 28 days after treatment, a significant increase (25–30%) in latency to respond to the heat stimulus was observed. Following a TMT dose of 5.25 mg/kg, an increase (30–60%) in latency was observed at 1, 14, 21, and 28 days. Howell et al. (44) also reported an increased latency (5- to 6-fold) to respond in Long-Evans rats treated with TMT (7 mg/kg) and tested on a 51°C hot plate. TMT did not affect

the peripheral nerve conduction velocity or its firing threshold as measured in the dorsala caudal nerve, but a decreased amplitude and an increased latency were observed in the early peaks of the somatosensory-evoked response recorded from this nerve. However, these changes in the evoked response were not dose related and must be viewed with caution. Finally, it should be noted that, although the authors interpret these findings to mean that the effects of TMT were centrally mediated, these results in and of themselves do not totally eliminate the possibility of a peripherally mediated effect.

Costa et al. (24) studied the effects of TMT on antinociception in albino (TEX:ICR) mice. Using a technique in which the tail was immersed into a 50°C water bath, dose-dependent antinociceptive effects were observed over a dose range of 1.06–4.26 mg/kg, i.p. The peak effect was observed at 2 h after 4.26 mg of TMT per kg and resulted in a more than 5-fold increase in the latency measure. This effect was not antagonized by naloxone, but it was completely antagonized by prior atropine administration at 10 mg/kg. In the same study, TMT was shown to produce a dose-related hypothermia. Following 4.26 mg of TMT per kg, body temperature dropped to approximately 32°C 2 h after administration and remained at that level for the remainder of the 6-h observation period. This finding is consistent with the study of Dyer et al. (34), who reported a dose-related hypothermia in Long-Evans rats following doses of 2.5, 5, or 10 mg of TMT per kg.

Dyer et al. (33) studied the effects of TMT on the functional integrity of the visual system. Rats treated with either 4, 5, 6, or 7 mg of TMT per kg showed dose-related changes in visually evoked responses that suggested retinal effects. These changes included an increase in latency and a decrease in the amplitude of the early peaks of the evoked response recorded from the visual cortex and the optic tract. Alterations in the late peaks of the evoked response which were characterized by both a decreased amplitude and decreased latency recorded from the visual cortex suggested that TMT also increased arousal in this area of the cortex.

As noted previously, many workers have reported an increase in spontaneous seizures following TMT, and there have been several studies which have examined seizure susceptibility in TMT-treated rats. Dyer and coworkers (31, 35) have used a kindling model for studying seizure susceptibility. Rats implanted with electrodes in either the amygdala or hippocampus and treated with either 5, 6 or 7 mg of TMT per kg kindled more rapidly than controls. Dyer et al. (35) also reported an increased seizure incidence in TMT-treated rats compared to controls when tested with either 30, 45, or 60 mg of pentylenetetrazol per kg.

In a study of the effects of TMT on chemically induced seizures in mice, Doctor et al. (28) administered 4.26 mg of TMT per kg, i.p., to mice and examined seizure susceptibility following bicuculine, isonicotinic acid hydrazide (INH), pentylenetetrazol, or strychnine at 1 and 14 h after TMT administration. At 1 h after TMT, a decrease compared to controls in seizure incidence was observed following convulsant doses of bicuculine, INH, and pentylenetetrazol. However, no protection was observed at 14 h after TMT. At 16 h after TMT, the mice exhibited spontaneous seizures and tremors followed by death. Little difference was observed in TMT-treated animals following convulsant doses of strychnine at 1 or 14 h post-TMT. Doctor and Fox (29) also reported on the effects of TMT in a maximal-electroshock seizure model in mice. TMT produced a dose-related decrease in seizure incidence and severity following electroshock at 0.5, 4, 21–24, and 96 h after TMT administration.

Several experimenters have administered TMT during prenatal and early neonatal periods and looked for behavioral effects later in life. In one such study, Ruppert et al. (83) administered either 0, 4, 5, or 6 mg of TMT hydroxide per kg to rat pups on postnatal day 5. In rats which received 5 or 6 mg of TMT per kg, a reduction in total body weight was observed at 21 days of age, as well as an impaired rope-climbing ability. When the same animals were tested at approximately 120 days of age, animals which received 5 and 6 mg/kg showed a decreased startle response, but only the 6-mg/kg animals showed a significantly different level of activity in a figure-8 maze. A significant increase in motor activity also was observed in these animals. Both the 5- and 6-mg/kg-treated rats showed a decreased whole brain weight, decreased olfactory bulb weight, and a decreased hippocampal weight when sacrificed at 120 days of age.

In another developmental study, Noland et al. (75) placed TMT in the drinking water of female rats 2 wk prior to breeding and allowed the chronic exposure to continue through breeding, gestation, and postnatal day 21. The concentration in the drinking water as tin was either 0.15, 0.5, or 1.0 mg/liter. No decrease in total liquid consumption by the dams was observed at any tin concentration. At 11 days of age, the pups were tested in a food-rewarded runway. Pups from the 1.0-mg/liter dams showed a decrease in acquisition of the runway task, while pups from dams exposed to 0.15 or 0.5 mg/liter were indistinguishable from controls. However, in an extinction test in the same runway, pups from all treatment groups showed a delay in extinction compared to pups from control dams. When the pups were tested in a swim-escape test at 21 days of age, no significant effects were observed in pups from the 0.15- and 1-mg/liter-exposed dams. Pups from dams exposed to 0.5 mg/liter showed a longer escape time than did controls.

2. *Conditioned behavior.* There have been several studies on the effects of TMT on conditioned behaviors. Walsh et al. (98) studied the effects of TMT on radial-arm maze performance in rats. Rats which received 6 mg of TMT per kg, p.o., made repeated entries into the same

arms of the radial maze and required 1.5–2 times the number of arm entries compared to saline controls to complete the task when tested at 15–35 days post exposure. These effects were still evident 49–70 days after dosing. In addition, the treated rats displayed a marked increase in activity compared to controls and to their own previous activity levels. Swartzwelder et al. (91) studied the effects of TMT on the performance of rats in a Hebb-Williams maze. As in the Walsh et al. (98) study, treated rats made a significantly greater number of errors than controls. In addition, treated rats showed a smaller error reduction, compared to controls, over a 10-day test period and displayed marked persevering behavior while running the maze.

TMT has been studied in several species and under several different conditioning procedures. Walsh et al. (96) studied the effects of TMT on passive avoidance behavior of rats. Male rats were intubated with a single dose of TMT (5, 6, or 7 mg/kg) and 21 days later were conditioned under a passive avoidance procedure. Treated rats exhibited a shorter latency to enter a compartment in which they had been shocked previously and shorter periods of immobility. Following 7 mg/kg, a greater than 6-fold reduction in latency and a nearly 50-fold reduction in periods of immobility were observed. These differences were not attributable to changes in foot shock sensitivity. Although significant differences compared to controls were demonstrated, no differences were observed between dose levels, so the data must be interpreted with caution.

MacPhail (60) studied the production of a conditioned flavor aversion in rats following administration of TMT. TMT produced a flavor aversion which depended on the dose and on the number of flavor-TMT pairings. The dose of TMT which reduced saccharin intake by 50% (ED_{50}) for TMT in the procedure was estimated to be 3.1 mg/kg.

Myers et al. (72) studied the effects of TMT on ethanol self-selection in rats given equal access to water and ethanol. Rats treated with 7 mg of TMT per kg consumed less ethanol than vehicle controls when tested at 21 and 150 days after TMT administration. This effect was observed at ethanol concentrations ranging from 3–30%. To test if the observed differences were related to a bitter taste, a quinine solution was substituted for ethanol. No differences in quinine intake were observed between control and TMT-treated rats. The difference in ethanol intake was also apparent under a food-contingent, schedule-induced polydipsia procedure.

TMT has been shown to produce significant effects on operant behavior which persist for long periods of time. Swartzwelder et al. (90) administered either saline or 7 mg of TMT per kg, p.o., to male Long-Evans rats, and 40 days later, the rats were food deprived, and lever pressing was established under a fixed-ratio (FR) schedule of food presentation. On the first day of testing, the FR requirement was set at FR 2. On successive days, the FR requirement was increased systematically up to an FR value of 99. At all FR values, TMT-treated rats were observed to have a higher lever pressing rate than saline-treated controls.

Wenger et al. (102) studied the effects of TMT on responding of two strains of mice under a mult FR FI schedule of food presentation. At a dose of 1 mg/kg, an approximate 15% decrease in the FI ¼-life value (a measure of the temporal pattern of FI responding) was observed 51 h after administration. This effect was observed in the C57BL/6N strain, but not in the BALB/c strain. At a dose of 3 mg/kg, a more pronounced behavioral effect was observed. At 3, 27, and 51 h after dosing in both strains, marked decreases were observed in both FR and FI response rates. At 51 h, FR responding was reduced to 10% of control, and FI responding was reduced to 14% of control values. FR responding returned to control values on days 5–9 after dosing and did not change after the ninth day. In contrast, FI responding showed a large increase (3-fold for C57BL mice and 1.5-fold for BALB/c mice) in responding on days 5–9. This increase in FI responding lasted for up to 6 wk in BALB/c mice and a somewhat shorter time in C57BL/6N mice. McMillan et al. (65, 66) have shown a qualitatively similar time course for the disruption of mult FR FI responding in pigeons following doses of 1, 1.75, and 2 mg of TMT per kg.

In another study using a schedule of temporally spaced responding in which only those responses separated by a 10- to 14-s period of no responding produced reinforcement, the effects of TMT were studied in rats (103). A single dose of 5.6, 7.5, or 10 mg/kg, i.p., produced dose-related decreases in the percentage of total responses spaced 10–14 s apart. At 7.5 mg/kg, this decrease in efficiency of responding (approximately 60% of control values) lasted approximately 2 wk. Following 10 mg/kg, a decrease to 30% of control values was seen during the first 14 days, and the efficiency was still markedly reduced 40 days after exposure. At both the 7.5- and 10-mg/kg doses, the decrease in efficiency of responding was associated with marked increases in number of responses. At 30–35 days after 10 mg/kg, the response rate was increased nearly 2.5-fold over control values. The majority of the responses occurred with an interresponse time of less than 4 s. Mastropaolo et al. (63) reported similar dose-related decreases in efficiency of responding in rats trained to respond under a DRL 15-s schedule of water presentation.

Under a matching-to-sample schedule which required the subjects to choose between two or more stimuli, one of which was identical to a stimulus presented immediately prior to a delay period during which no stimuli were presented, TMT decreased the matching accuracy of both pigeons (50) and rhesus monkeys (12). In pigeons, decreases in matching accuracy frequently occurred at

doses having little effect on rate of responding. As with behavioral effects of TMT in other species under other procedures, the effects of TMT on matching-to-sample performance in pigeons and monkeys were of long duration.

B. Tissue Distribution of TMT

There have been a few studies which have examined tissue levels of TMT following administration to laboratory animals. Doctor et al. (30) administered 4.26 mg of TMT per kg, i.p., to adult male mice. A peak in tissue levels was observed at 1 h after TMT administration in the kidney, liver, blood, lungs, and testes. However, in the brain, skeletal muscle, and adipose tissues, a peak was not observed until 16 h after administration. At 16 h, the rank order and tissue concentrations (μg/g tissue) were: liver, 4.78; testes, 3.40; kidney, 2.51; lung, 1.73; brain, 1.53; skeletal muscle, 1.37; adipose tissue, 1.00; and blood, 0.82.

Brown et al. (11) examined blood and brain tissue levels at various doses and times after administration of TMT chloride in adult male Wistar rats. In contrast to mice, at all doses and time periods, higher levels of tin were found in blood than in brain tissue. At 24 h after TMT chloride, 5 mg/kg, i.v., the concentration of TMT in brain tissue was 1.44 μg/g of wet tissue, while the concentration in blood was 5.16 μg/g. This high concentration in blood appears to be associated with erythrocytes, since plasma sampled at the same time contained only 1.12 μg/ml. Results following multiple dosings at 7-day intervals indicated a similar ratio of blood to brain tissue concentrations.

Cook et al. (23) have examined the subcellular distribution of TMT in rat brain tissue following administration of TMT, 6 mg/kg. Maximal levels were achieved within 12 h after exposure for all subcellular fractions except the P_2 fraction (myelin, synaptosome, and mitochondria). Concentrations of tin did not reach maximal levels in the P_2 fraction until 5 days after exposure. In the same study, TET peak concentrations were observed in the P_2 fraction within 24 h after TET administration.

Mushak et al. (71) examined tissue concentrations in the developing rat exposed to different doses of TMT from postnatal days 2–29. Rat pups exposed to TMT hydroxide, 1 mg/kg/day, and sacrificed on day 30 had a rank order of tissue levels of blood $\gg$ liver $>$ kidney $>$ brain. Total tin in blood was determined as 3.15 μg/g, while brain tissue contained 72 ng/g of tissue. These values indicate a ratio of blood to brain concentrations similar to that observed in adult rats (11).

C. Effects of TMT on the Nervous System

1. TMT and nerve damage. The neuropathology of TMT has been studied in several species, most extensively in the rat and mouse. In both C57BL and BALB/c mice, little pathology was observed at doses below 3 mg/kg. At 51 h after 3 mg of TMT per kg, a significant amount of neuronal necrosis was observed in the fascia dentata of both strains. The degree of neuronal necrosis declined by the ninth day after exposure, and no necrotic cells were visible 23 days after exposure. At this latter time, some thinning or atrophy of the fascia dentata was observed, suggesting a reduction in total cell numbers (101, 102). Chang et al. (17, 19) studied the neuropathology produced by TMT in BALB/c mice which received TMT chloride, 3 mg/kg, 48 h prior to sacrifice. At the light microscopic level, neuronal necrosis was evident not only in the fascia dentata of the hippocampus but also in the neocortex, pyriform cortex, amygdala nucleus, and brain stem. In the hippocampus, very little if any necrosis was observed in the pyramidal cells of Ammon's horn. The damage in the brain stem was most prominent in the large neurons, particularly those in the mesencephalic trigeminal nuclei. While light microscopic examination of the hippocampus indicated a relative sparing of the pyramidal cells, electron microscopic examination indicated extensive accumulation of lysosomes in both the neuronal bodies and processes of these cells. An intracellular edema was a prominent feature of both cell types of the hippocampus. As a result of the edema, vacuolation was significant (20).

In a detailed study of the brain stem pathology produced by 3 mg of TMT per kg in BALB/c mice, Chang et al. (18) reported degenerative and vacuolar changes in the neurons of the mesencephalic trigeminal nuclei at 48–51 h after TMT. These neurons had a chromalytic character, eccentric nuclei, a loss of Nissl substance, and a distention of the endoplasmic reticulum and Golgi complex. The distention produced by intraneuronal vacuolation reflects an intracellular edema.

Chang et al. (21) also studied changes produced in the spinal cord of C57BL mice exposed to TMT chloride, 3 mg/kg, 48–72 h prior to sacrifice. The most significant changes were observed in the medial and lateral motor nuclei of the anterior horn of the spinal cord. These neurons showed chromalytic and vacuolar changes with lysosomal accumulation and extensive dilation of the cytoplasmic membranes (endoplasmic reticulum and Golgi complex). Large intraneuronal vacuoles were also present.

This pattern of neuropathology produced by TMT in adult mice is not in total agreement with the neuropathology produced by TMT during early postnatal periods. Reuhl et al. (80) reported that 16 h after 3 mg/kg were administered to 3-day-old BALB/c mice, a significantly increased eosinophilia and granularity were observed in the pyramidal cells of the hippocampus and in the neurons of the pyriform cortex. From 1–10 days after 3 mg/kg, degeneration and necrotic changes were evident in region CA_3 of the hippocampus, and the changes observed in the fascia dentata were much less than those observed in adult mice.

In the rat, the neuropathology produced by TMT is,

like the mouse, largely restricted to the neuron. Brown et al. (11) in an extensive study of TMT in male Wistar rats reported no observable pathology at times ranging from 1–70 days after exposure in the retina, optic nerve, lumbar spinal ganglia, and sciatic nerves following single or four separate administrations of 2 or 4 mg/kg, or after a single dose of 10 mg/kg. In two rats killed 1 day after a single dose of 10 mg of TMT chloride per kg, no pathology was observed. At 2 days after this dose, minimal damage was observed in both the pyramidal cells and the fascia dentata cells of the hippocampus. Minor changes characterized by a loss of Nissl substance and an increased eosinophilia of the cytoplasm were also observed in the pyriform cortex and amygdala. In rats sacrificed at 3–4 days after dosing, damage was reported to neurons of the neocortex, pyriform cortex, and amygdaloid nucleus, in addition to more extensive damage in the pyramidal cells and fascia dentata cells of the hippocampus. The peak in observable pathology occurred at 10–20 days after the 10-mg/kg dose of TMT chloride.

Chang and Dyer (15) studied the time course of TMT chloride in adult male Long-Evans rats. As in the Brown et al. (11) study, necrotic neurons were first observed in the hippocampus at 3 days after a single dose of TMT chloride (7.5 mg/kg). Necrotic neurons were also observed in the pyriform/entorhinal cortices and olfactory tubercle. At 15 days after exposure, extensive neuronal necrosis and astriogliosis were observed in these same areas. By 60 days after exposure, the pyramidal neurons of Ammon's horn showed an extensive cell loss. The most vulnerable cells appeared to be those in region CA_3 (anteromedial or septal portion of the horn). Chromalytic changes were also observed in the large mesencephalic trigeminal neurons. Interestingly, the granule cells of the hippocampus showed little damage at this dose of TMT.

Similar observations of neuropathology following TMT administration in rats have been reported by Dyer et al. (34) and Ruppert et al. (84). In addition, Dyer et al. (32) proposed that a simple measure of the degree of damage induced by TMT could be obtained by measuring the length of the line of pyramidal cells from CA_2 through CA_{3c}. The loss of pyramidal cells appeared to begin in CA_{3c}, progressing through CA_3 and CA_{3a} in a dose- and time-dependent manner.

Bouldin et al. (9) studied the time course of TMT-induced neuropathology in adult Long-Evans rats at the electron microscopic level. Rats were administered TMT hydroxide, 5 mg/kg/day, and sacrificed 24 h after one, two, three, four, or five daily doses. No significant changes were observed at 24 h after the first dose of TMT. At 24 h after the second dose, significant cytoplasmic changes were observed in neurons of the fascia dentata and Ammon's horn of the hippocampus. Multifocal accumulations of smooth unit membranes were observed in the cytoplasm of perikaryon and proximal dendrites. The membranes in the form of vesicles and tubules formed electron-dense material in the cytoplasm. Vacuoles were also observed in those neurons which contained these electron-dense vesicles and tubules. At 24 h after the third daily injection, the first necrotic neurons were evident in the hippocampus. The remaining viable cells contained a variety of dense-cored vesicles and tubules. These dense-cored vesicles in the neuronal perikarya and proximal dendrites were the most striking abnormality at this stage of intoxication. In rats receiving four doses of TMT and sacrified 24 h after TMT, necrotic neurons were quite evident in the fascia dentata and Ammon's horn of the hippocampus. The nuclei of these neurons were frequently shrunken and electron dense. Cytoplasmic processes of macrophages were starting to engulf the dead neurons. In the remaining viable cells of the rats which received four doses of TMT, electron-dense bodies were numerous, and autophagic vacuoles were evident. Mitochondria appeared relatively normal, but a decrease was observed in the amount of apparent rough endoplasmic reticulum.

In the same study (9), the effects of TMT were determined in neonatal rats receiving chronic doses of TMT. A dose of TMT·OH, 1 mg/kg/day, was given on alternate days from days 3–29 of life. Upon sacrifice at day 30, a similar pattern of neuropathology was observed as seen in adult rats.

A different approach to neonatal exposure has been reported by Chang (14). Groups of neonatal rats were administered a single dose of 6 mg/kg on selected days ranging from postnatal days 1–30. The resultant neuropathology produced correlated well with the morphological development of the hippocampal formations at the time of exposure. No pathology was observed in rat pups exposed to a single dose between postnatal days 1 and 4. However, animals exposed on days 5 or 6 showed lesions in area CA_{3b}; animals exposed on day 7 had significant pathology in areas CA_{3a} and CA_{3b}; on days 8, 9, or 10, lesions were evident in the entire Ca_3 region (*a*, *b*, and *c*); with dosing on days 11 or 12, areas CA_2 and CA_3 were affected; and finally, with dosing on days 13, 14, or 15, the entire Ammon's horn (Ca_1, CA_2, and CA_3) was shown to be affected. Dosing after postnatal day 20 produced pathology similar to that seen in adult rats. Thus, the lesion produced correlates well with the developing functional maturity of cell types within the hippocampus. This would suggest that the toxicity of TMT on pyramidal cells may not be just a direct effect of TMT on the pyramidal cells, but the possibility exists that the damage to the cells is indirectly mediated through an altered functional interaction between cell types of the hippocampus.

The neuropathology of TMT has also been studied in the marmoset (1) and the pigeon (65, 66). The pathology in the marmoset more closely resembles the effects observed in the mouse than the rat. Specifically, a dose of 3 mg/kg produces extensive neuronal necrosis in the

fascia dentata of the hippocampus, amygdaloid nucleus, neocortex, and pyriform cortex. In the pigeon, 1.75 mg of TMT per kg produces almost total loss of hippocampal neurons, as well as neuronal damage in the brainstem and striatum griseum centrale.

The issue of species and strain comparisons has been addressed specifically in a study by Chang et al. (22). In this study, the effects of TMT were directly compared in BALB/c and C57BL/6N mice and Long-Evans and Sprague-Dawley rats. Mice were given TMT·Cl, 3 mg/kg, and rats were given a functionally equally effective dose, 7 mg/kg. Animals were sacrificed at the time of onset of observable neurological or behavioral (tremor, aggression) signs, 48 h for mice, 72 h for Long-Evans rats, and 120 h for Sprague-Dawley rats. In general, the mice showed more prominent neurological symptoms and neuropathology than the rats, and Long-Evans rats were more sensitive than Sprague-Dawley rats. Mice had prominent lesions in the hippocampal fascia dentata, brain stem, and spinal cord. In contrast, rats had only minor damage to the fascia dentata, and prominent lesions were observed in the olfactory cortices and in the Ammon's horn of the hippocampus.

While TMT has relatively little effect on peripheral nerves, Chang and Dyer (16) examined the effects of 6 mg of TMT per kg on sensory nerves of adult male Long-Evans rats. At 72 h after exposure, a swelling was observed in the optic fiber layers of the retina, as well as necrotic changes in the ganglion and inner nucleus layers of the retina. At later time periods, the retina was relatively devoid of ganglia. The inner ear was also sensitive to the effects of TMT. Within 24 h after administration of TMT, an edematous swelling was observed in the hair cells of the inner ear, and vacuolar changes were evident in the spiral ganglion cells of the organ of Corti. At 15 and 30 days after exposure, extensive destruction of these same structures was evident. At 15 days after exposure, necrotic changes were observed, as noted in other reports, in both the pyriform cortex and olfactory tubercle. Thus, evidence of sensory impairment exists in TMT-treated animals.

2. TMT and neurotransmitters. TMT has been shown to produce a number of significant effects in neurotransmitter systems. Valdes et al. (94) administered three doses at weekly intervals of either 0, 2, 3, or 4 mg of TMT chloride per kg to adult male Long-Evans rats. The rats were sacrificed at 4 days after the third dose of TMT. Uptake of the neurotransmitter, GABA, by brain tissue showed an increased affinity (V_{max}) and decreased capacity (K_d), but the uptake of norepinephrine showed a reduced affinity and an increased capacity of uptake.

DeHaven et al. (25) examined the effect of TMT chloride on dopamine and serotonin (5-HT) systems. A dose of 7 mg/kg administered 7 days before sacrifice decreasd levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in the nucleus acumbens. At a dose of 3 mg/kg, the effects on dopamine and DOPAC were marginal. No effects on dopamine or DOPAC levels were seen with either dose in the striatum, olfactory tubercle, septum, amygdaloid or pyriform cortex. Following 3 mg/kg TMT, a reduction in 5-HT was observed in the striatum and nucleus acumbens and an increase in 5-hydroxyindoleacetic acid (5-HIAA) was observed in the hippocampus. At 7 mg/kg TET, 5-HT levels were decreased in the amygdaloid and pyriform cortex, and 5-HIAA levels were increased in the striatum, nucleus acumbens, septum, amygdaloid, pyriform cortex and hippocampus. When turnover was estimated for dopamine and serotonin by determining the ratio of DOPAC/dopamine and 5-HIAA/5-HT, respectively, 5-HT turnover was increased in the amygdaloid and pyriform cortex at 3 mg/kg and in all brain regions at 7 mg/kg. No change was observed in dopamine turnover following either dose in any brain region. Likewise, no change was observed in dopamine receptor binding (H^3-spiperone binding).

When TMT hydroxide was administered on alternate days beginning on postnatal day 2 and continuing through day 28, and the rats were sacrificed on day 55, GABA levels were reduced in the hippocampus and unchanged in the cortex and hypothalamus. Dopamine levels were reduced in the striatum but not affected in the brain stem. No change in DOPAC was observed in the striatum. No change was observed in norepinephrine in the brain stem or cerebellum, and acetylcholine was unaffected in the cortex, hippocampus and hypothalamus (61).

In 8 to 10-wk-old mice given either a single p.o. dose of 1 or 3 mg of TMT hydroxide per kg and sacrificed at 2, 7, and 14 days after exposure, a decrease in whole brain levels of norepinephrine was observed at 2 days after both doses of TMT, but no effect was observed at 7 and 14 days. The decrease observed at 2 days after 3 mg/kg was associated with an increase in levels of homovanillic acid (HVA). These changes were not associated with any significant changes in norepinephrine receptor binding. In contrast, acetylcholine receptor binding was decreased at 2 and 7 days after exposure, and dopamine receptor binding was increased at 7 and 14 days (3).

D. Summary of TMT Effects

The effects of TMT in laboratory animals are observed over an unusually narrow dose range. The dose-response curves are unusually steep, and the difference between doses producing no observable effects and doses producing 100% lethality is approximately 10-fold in all species and, in some species, considerably less. TMT produces a variety of changes in the gross behavior. The most consistent effect reported in all species is a marked whole body tremor. In addition, a variety of changes, including seizures, tail chasing and mutilation, vocalizations, and increases in aggressive behavior, were reported in one or more species. Using behavioral test procedures such as spontanous motor activity, open field activity, maze per-

formance, and a variety of different schedules of reinforcement, significant differences were reported in the time course of the behavioral effects of TMT compared to TET. An initial decrease in the frequency of most behaviors is reported to occur in most species following TMT. This decrease paralleled the time course of the apparent health of the animals and peaked 3–5 days after TMT administration. However, the duration of the behavioral disruption frequently did not parallel the return of apparent state of health of the animals, and behavior remained disrupted for long periods of time. TMT has also been shown to disrupt normal sensory function, resulting in altered pain perception and altered visually evoked potentials.

The tissue distribution of TMT shows a marked species difference. In mice, TMT concentrations in a variety of tissues including the brain exceeded the levels found in plasma. In contrast, in the rat, blood levels of TMT exceeded all other tissues. The high blood levels of TMT in the rat appear to be associated with erythrocytes.

The neuropathology produced by TMT is largely restricted to neurons. In the adult mouse, evidence of neuropathology is evident at the light microscope level in the fascia dentata of the hippocampus, neocortex, pyriform cortex, amygdaloid nucleus, and brain stem. Neuropathology in the rat following TMT is first observed in the hippocampus followed by neuronal damage in the pyriform/enterorhinal cortices, olfactory tubercle, and in the large mesencephalic trigeminal neurons. It is interesting that the pyramidal neurons and the fascia dentata of the hippocampus show a marked species difference with respect to TMT. In both the rat and mouse, neonatal exposure produced a different pattern of neuropathology compared to the adult exposure, and the exact pattern of pathology was dependent upon the age of the animal at the time of exposure. TMT exposure in the rat also produced damage to sensory neurons and structures which may explain some of the sensory impairment reported following TMT. A variety of neurochemical changes have also been reported in the rat brain following exposure to TMT. These changes involve the neurotransmitters, norepinephrine, dopamine, 5-HT and GABA.

Although it is tempting to speculate on the cause and effect relationship between the neuropathology produced by TMT and the behavioral changes observed, it is a very difficult, if not impossible, task to prove such a relationship. However, it should be noted that much of the neuropathology does show a similar time course to the onset of behavioral deficits. The correlation between the duration of behavioral deficit and pathology is a much harder task and requires accurate estimates of total surviving cell counts, etc. Such data currently are not available.

IV. Neurobehavioral Toxicology of Alkyltins: Problems and Promises

Our knowledge about the neurobehavioral toxicology of trialkyltins can be summarized in a few sentences. Both trialkyltins produce both behavioral and morphological changes. Morphologically, TET is associated with edema and myelin splitting in the CNS. These changes appear to disappear slowly with the passage of time. The behavioral changes have a rapid onset, but they appear for the most part to be reversible in surviving animals. TMT is associated with neuronal death. The effects of TMT on behavior appear to be somewhat slower in onset and much longer in duration, perhaps in some cases being irreversible. Although such statements represent a beginning point, they are hardly adequate descriptions of the neurobehavioral toxicity of these chemicals, much less being very informative about mechanisms involved. As one tries to understand the data beyond such simple statements, the major problems that plague all of neurobehavioral toxicology become apparent. Some of these problems will be discussed briefly in the context of alkyltin toxicity.

The first problem one encounters in describing the effects of alkyltins is a technical difficulty in making comparisons across studies due to the different alkyltin salts and different ways that doses have been specified in different studies. TET has generally been administered as the chloride, bromide, or sulfate salt, although there are a few instances where the hydroxide salt has been used. TMT has generally been given as the chloride or hydroxide salt. Some investigators have calculated their doses in terms of mg of the salt per kg, while others have calculated doses in terms of the alkyltin without the linked ion. In a few instances, the dose has been specified in tin content, disregarding both the ion and the alkyl groups. For TET, the alkyltin content of these commonly used salts varies from 72–92% and the tin content from 42–53%. For TMT, the alkyltin content of the chloride and hydroxide salts ranges from 82–91% and the content of tin from 60–66%. Thus, in an extreme case, 1 mg of the TET hydroxide dose actually contains about 0.2 mg more TET than a 1-mg TET bromide dose, or about 28% more TET in the hydroxide salt.

For pharmacologists and toxicologists accustomed to working over 3- to 10-fold dosage increments in calculating dose-effect curves, dosage differences of 28% or less may appear trivial, but the steepness of the dose-effect curves for TET and TMT can create problems in making comparisons across studies with different alkyltin salts. For example, Ruppert et al. (84) administered TMT as the chloride salt and calculated their doses in terms of the alkyltin without the chloride ion. Doses of 5 and 6 mg/kg were without significant effect on the activity in a figure-8 maze, while a 7-mg/kg dose produced large increases. A 7-mg/kg dose of TMT calculated as the chloride salt would contain less than 6 mg of alkyltin per kg, which is not in the effective range reported in the study of Ruppert et al. (84).

Those investigators who have reported their doses in terms of alkyltin content have largely avoided such prob-

lems, unless they wish to make comparisons across alkyltin compounds on the basis of tin content. Those investigators who have calculated their doses in terms of the salt can at least make dosage comparisons with other studies after making the appropriate dosage conversions, although it places an unnecessary burden on those reading the literature. Unfortunately, it is impossible to make dosage comparisons across some studies, since the dosage form is not specified. In fact, some studies fail to mention either the salt used or the basis on which the dose was calculated. When listing doses in this review, the authors did not attempt to convert all doses to a common base, both because of the labor involved and because of the large number of important studies that would have to be excluded because such conversions were impossible. In the few instances in which potency comparisons have been made, care has been taken to make sure the potency differences are sufficiently large to override small differences in dose due to the way the dose was calculated, or to make sure that the comparisons were based on the same dosing specifications.

Another problem is describing the specificity of behavioral effects produced by alkyltins. Many drugs appear to have reasonably specific effects on behavior. For example, amphetamines have rate-dependent effects on schedule-controlled responding, further decrease punished responding, increase locomotor activity, produce stereotyped behavior, and so on (68). Similar generalizations could be developed for many other drug classes, but they are difficult to make with alkyltin compounds. With the possible exception of the effects of TMT on the temporal pattern of responding under DRL schedules and the motor activity-increasing effects of TMT, there are few generalizations that can be made about specific behavioral effects of alkyltins. These compounds appear to be rather nonspecific behavioral toxicants, regardless of the behavioral measure considered.

There are similar concerns about the specificity of neuropathological effects of alkyltins. For example, early descriptions of the neuropathology of TMT pointed to neuronal death in the hippocampus (9, 11). Subsequently, many investigators began to look upon TMT lesions as a model for producing hippocampal damage and as a tool for investigating the functional role of the hippocampus. Unfortunately, subsequent studies have indicated that the neuronal damage produced by TMT is considerably more widespread than recognized initially (15, 21). In fact, TMT appears to produce damage in a variety of neuronal systems in the CNS. The apparent specificity of TMT in the hippocampus was based at least in part on a failure of investigators to look for damage at other sites following the initial descriptions of damage in the hippocampus.

The initial descriptions of the behavioral effects of alkyltins and the apparently specific sites for lesions in the brain encouraged many investigators to take one step further, that is, to suggest cause and effect relationships between the observed neuropathology and a specific behavioral effect. As Wenger et al. (103) have pointed out, suggestions about cause (neuropathology) and effect (behavior) relationships generally have been based on weak correlations. Even a high correlation between neuropathology and behavioral change does not establish a cause and effect relationship between brain damage at specific sites and particular behavioral changes, especially when an investigator arbitrarily picks an isolated behavioral effect and then refers to neuropathology reports in the literature published by another investigator to explain the behavioral effect.

In order to establish cause and effect relationships between the neuropathological changes produced by alkyltins and behavior, considerably more sophisticated research needs to be done than that generally being pursued by investigators today. For example, it would be necessary to quantify a large range of behavioral and neuropathological changes produced by an alkyltin compound. This means carefully quantifying the neuropathology using cell counts and other objective measurements rather than the simple observational impressions frequently reported by neuropathologists who, in many cases, are not blind to the treatment conditions. It means an investigation of a wide range of behaviors that have also been objectively measured for a range of doses of each compound under investigation. The performance of such parametric studies will produce correlations between particular types of neuropathology and specific behaviors, or groups of behaviors. With such correlations established, we can begin to confirm cause and effect relationships using the classical techniques for studying brain-behavior relationships. For example, do similar types of lesions produced by other methods produce behavioral effects similar to those produced by alkyltins? Do agents that prevent the lesions (assuming such agents exist) also prevent the behavioral changes?

Even if one makes only limited statements about cause and effect relationships between neuropathology and behavioral changes produced by alkyltins, major difficulties remain. For example, McMillan et al. (67) recently reported on the behavioral effects of several doses of TET and TMT over a 16-day period following administration. The effects of TMT on lever pressing for food pellets, water drinking, and running in an activity wheel were complex, with initial decreases in behavior produced by TMT gradually being replaced by marked increases in rates of drinking, lever pressing, and running. When the animals were sacrificed and the neuropathology determined, the investigators were faced with the problem of what behavioral change to correlate with what neuropathological observation. The neuropathology was necessarily determined at a fixed point in time, but the behavioral effects occurred differentially during the previous 16 days. Should one attempt to correlate the neu-

ropathology with the initial TMT effect, the effect that occurred in closest temporal proximity to the time of animal sacrifice, the peak behavioral effect produced by TMT, the most frequently observed effect, or some cumulative measure of all TMT effects on behavior? The answeres to such a question are not obvious. It could be argued that the answer to the question is "all of the above." Thus, a large number of animals could be trained with serial sacrifices being made for neuropathology. Perhaps, this is the only answer, yet the prospect of extended training of large numbers of animals that will be sacrificed within a short period after alkyltin administration is not attractive to many investigators and will be expensive and time consuming.

Frequently, investigators have observed instances where behavioral effects of alkyltins preceded neuropathological changes, or where neuropathology could be observed at times when there were no behavioral effects. This does not mean that the neuropathology is unrelated to the behavior any more than the correlation between behavior and neuropathology establishes cause and effect. For example, TET produces behavioral effects within an hour after injection in mice, yet edema and myelin splitting cannot be detected until a later period in time. This does not prove there is no relationship between cerebral edema and changes in behavior, as it is quite likely that those events antecedent to the production of the edema are producing the behavioral changes and that the behavioral changes are apparent before sufficient edema occurs that can be detected. Perhaps, at the level of electron microscopy, earlier morphological changes will be observed.

Similarly, we have observed neuropathology in animals that have shown complete behavioral recovery. This does not necessarily imply that the original lesion was not responsible for the behavioral changes, since it is widely recognized that other systems in the brain can assume the functions of damaged systems, especially when continued training of the organism occurs.

Like much of the rest of toxicology, neurobehavioral toxicology has been influenced by government needs for regulatory laws governing toxic chemicals in the environment. For example, the Toxic Substances Control Act emphasizes neurobehavioral testing of chemicals. Although the need for such laws is not debatable, there have been some unfortunate consequences. Perhaps the most unfortunate consequence has been a competition between neurotoxicologists and behavioral toxicologists to have their respective disciplines featured in the regulatory rules. Two disciplines that should be cooperating closely in describing and explaining the consequences of the interaction between toxic chemicals and the nervous system have devoted too much time trying to show that one approach is more "sensitive" than the other to the effects of toxic chemicals or a more "valid predictor" of the consequences of human exposure. From a scientific viewpoint, such competition makes little sense.

Regulatory laws have emphasized determinations of exposure levels unlikely to produce serious risks with chronic exposure. This emphasis has also influenced the direction of development of neurobehavioral toxicology. Whereas behavioral pharmacology has emphasized the acute effects of drugs and largely ignored their chronic effects, behavioral toxicology has concentrated on studies of the effects of chronic exposure to low levels of toxic chemicals. Although chronic exposures are of great practical importance, a consequence of this emphasis has been that behavioral toxicologists have frequently worked with the chronic administration of acutely ineffective doses before adequately describing the acute effects of larger doses.

Any toxicological study used for regulatory purposes raises the issue of extrapolation to man. Such problems are by no means unique to behavioral toxicology, although many toxicologists seem to think they are. Many scientists are willing to accept the results of a test of the effects of a compound on isolated cells in culture as evidence of potential toxicity in humans. The fact that the test was not carried out in an intact animal or even in human tissue usually is not at issue. What is important is that the data can stand up to the tests of reliability and predictive validity and show clear evidence of an effect of the compound being tested. Yet, the same people are not willing to use the same criteria for behavioral testing. Instead of discussing the reliability and predictive validity of the data, the issue of the "importance" or "meaning" of behavioral effects in animals is discussed. Unfortunately, the losers in such issues are the general public whose welfare we are trying to protect. Is not the demonstration of the ability of a compound to alter behavioral states an important consideration in determining what a "safe" exposure level should be for workers operating heavy equipment or piloting a 747 airplane? When neurobehavioral toxicology is subjected to the same rigorous examination of its reliability and predictive validity as any other type of toxicity testing, neurobehavioral toxicology should compare well with most other types of testing.

A further difficulty established by the emphasis on the regulatory and safety aspects of toxicity testing is the reduction of scientific inquiry to a "pass-fail" approach to research and a "toxic substance of the month" emphasis. Interest in the neurobehavioral toxicity of a substance of environmental or commercial concern has been rather limited to the establishment of a safe exposure limit, and there has been little interest in compounds which are of no environmental or commercial concern. For example, several years ago a great deal of interest was shown in the alkyltins. They were used in industry and were shown to be potent neurotoxins. Recently, because of the now documented neurotoxicity, the com-

mercial use of alkyltins has dropped dramatically, and a parallel decrease is beginning to occur in the number of investigators actively studying the neurotoxicity of this group of compounds. The message for many investigators is that only those compounds which are of environmental or commercial importance are to be studied. However, toxic substances historically have been used to study the nervous system. Compounds such as curare, nicotine, and tetrodotoxin have told us much about how the peripheral nervous system functions. In a similar fashion, substances which have specific neurotoxic effects in the central nervous system, such as the alkyltins, may have greater potential for determining basic function and organization of the central nervous system than has been realized by the years of study with therapeutic compounds. Certainly the scientific contribution of toxicology, and specifically neurobehavioral toxicology, will be greater than a mere determination of whose test is the most sensitive or what is the minimum safe level of exposure. These are indeed important issues, but they should not be the major contribution of the discipline.

Acknowledgments. We wish to thank Marjorie A. McMillan for major assistance with the literature search and Brenda K. Selby for typing the manuscript. Although some of the research by the authors was supported by the United States Environmental Protection Agency under assistance agreement number R809452-01 to the University of Arkansas for Medical Sciences, the review does not necessarily reflect the view of the Ageny, and an official endorsement should not be inferred.

REFERENCES

1. ALDRIDGE, W. N., BROWN, A. W., BRIERLEY, J. B., VERSCHOYLE, R. D., AND STREET, B.W.: Brain damage due to trimethyltin compounds. Lancet **2:** 692–693, 1981.
2. ALEU, F.R., KATZMAN, R., AND TERRY, R. D.: Fine structure and electrolyte analysis of cerebral edema induced by alkyltin intoxication. J. Neuropathol. Exp. Neurol. **22:** 403–413, 1963.
3. ALI, S. F., CRANMER, J. M., GOAD, P. T., SLIKKER, W., HARBISON, R. D., AND CRANMER, M. F.: Triemethyltin induced changes of neurotransmitter levels and brain receptor binding in the mouse. Neurotoxicology **4:** 29–36, 1983.
4. AMOCHAEV, A., JOHNSON, R. C., SALAMY, A., AND SHAH, S. N.: Brainstem auditory evoked potentials and myelin changes in triethyltin-induced edema in young adult rats. Exp. Neurol. **66:** 629–635, 1979.
5. BARNES, J. M., AND STONER, H. B.: Toxciology of tin compounds. Pharmacol. Rev. **11:** 211–231, 1959.
6. BIERKAMPER, G. G.: In vitro assessement of neuromuscular toxicity. Neurobehav. Toxicol. Teratol. **4:** 597–604, 1982.
7. BIERKAMPER, G. G., AND VALDES, J. J.: Triethyltin intoxication alters acetylcholine release from rat phrenic nerve-hemidiaphragm. Neurobehav. Toxicol. Teratol. **4:** 251–254, 1982.
8. BLAKER, W. D., KRIGMAN, M.R., THOMAS, D. J., MUSHAK, P., AND MORELL, P.: Effect of triethyl tin on myelination in the developing rat. J. Neurochem. **36:** 44–52, 1981.
9. BOULDIN, T. W., GOINES, N. D., BAGNELL, R. C., AND KRIGMAN, M. R.: Pathogenesis of trimethyltin neuronal toxicity. Ultrastructural and cytochemical obeservations. Am. J. Pathol. **104:** 237–249, 1981.
10. BRODY, T. M., AND MOORE, K. E.: Biochemical aspects of triethyltin toxicity. Fed. Proc. **21:** 1103–1106, 1962.
11. BROWN, A. W., ALDRIDGE, W. N., STREET, B. W., AND VERSCHOYLE, R.D.: The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat. Am. J. Pathol. **97:** 59–82, 1979.
12. BUSHNELL, P. J., AND EVANS, H. L.: Trimethyltin-induced memory impairment in nonhuman primates. Fed. Proc. **43:** 763, 1984.
13. BUSHNEL, P. J., AND EVANS, H. L.: Effects of trimethyltin on homecage behavior of rats. Toxicol. Appl. Pharmacol. **79:** 134–142, 1985.
14. CHANG, L. W.: Trimethyltin induced hippocampal lesions at various neonatal ages. Bull. Environ. Contam. Toxicol. **33:** 293–301, 1984.
15. CHANG, L. W., AND DYER, R. S.: A time-course study of trimethyltin induced neuropathology in rats. Neurobehav. Toxicol. Teratol. **5:** 443–459, 1983.
16. CHANG, L. W., AND DYER, R. S.: Trimethyltin induced pathology in sensory neurons. Neurobehav. Toxicol. Teratol. **5:** 673–696, 1983.
17. CHANG, L. W., TIEMEYER, T. M., WENGER, G. R., AND MCMILLAN, D. E.: Neuropathology of mouse hippocampus in acute trimethyltin intoxication. Neurobehav. Toxciol. Teratol. **4:** 149–156, 1982.
18. CHANG, L. W., TIEMEYER, T. M., WENGER, G. R., AND MCMILLAN, D. E.: Neuropathology of trimethyltin intoxication. III. Changes in the brain stem neurons. Environ. Res. **30:** 399–411, 1983.
19. CHANG, L. W., TIEMEYER, T. M., WENGER, G. R., MCMILLAN, D. E., AND REUHL, K. R.: Neuropathology of trimethlytin intoxication. I. Light microscopy study. Environ. Res. **29:** 435–444, 1982.
20. CHANG, L. W., TIEMEYER, T. M., WENGER, G. R., MCMILLAN, D. E., AND REUHL, K. R.: Neuropathology of trimethyltin intoxication. II. Electron microscopic study on the hippocampus. Environ. Res. **29:** 445–458, 1982.
21. CHANG, L. W., WENGER, G. R., AND MCMILLAN, D. E.: Neuropathology of trimethyltin intoxication. IV. Changes in the spinal cord. Environ. Res. **34:** 123–134, 1984.
22. CHANG, L. W., WENGER, G. R., MCMILLAN, D. E., AND DYER, R. S.: Species and strain comparison of acute neurotoxic effects of trimethyltin in mice and rats. Neurobehav. Toxicol. Teratol. **5:** 337–350, 1983.
23. COOK, L. L., HEATH, S. M., AND O'CALLAGHAN, J. P.: Distribution of tin in brain subcellular fractions following the administration of trimethyl tin and triethyl tin to the rat. Toxicol. Appl. Pharmacol. **73:** 564–568, 1984.
24. COSTA, L. G., DOCTOR, S. V., AND MURPHY, S. D.: Antinociceptive and hypothermic effects of trimethyltin. Life Sci. **31:** 1093–1102, 1982.
25. DEHAVEN, D. L., WALSH, T. J., AND MAILMAN, R. B.: Effects of trimethyltin on dopaminergic and serotonergic function in the central nervous system. Toxicol. Appl. Pharmacol. **75:** 182–189, 1984.
26. DEHAVEN, D. L., WAYNER, M. J., BARONE, F.C., AND EVANS, S. M.: Effects of triethyltin on ingestive behavior at ad lib, reduced, and recovered body weight. Neurobehav. Toxicol. Teratol. **4:** 217–223, 1982.
27. DEHAVEN, D. L., WAYNER, M. J., BARONE, F. C., AND EVANS, S. M.: Effects of triethyltin on schedule dependent and schedule induced behaviors under different schedules of reinforcement. Neurobehav. Toxicol. Teratol. **4:** 231–239, 1982.
28. DOCTOR, S. V., COSTA, L. G., AND MURPHY, S. D.: Effect of trimethyltin on chemically-induced seizures. Toxicol. Lett. **13:** 217–223, 1982.
29. DOCTOR, S. V., AND FOX, D. A.: Effects of organotin compounds on maximal electroshock seizure (MES) responsiveness in mice. I. Tri (n-alkyl)tin compounds. J. Toxicol. Environ. Health **10:** 43–52, 1982.
30. DOCTOR, S. V., SULTATOS, L. G., AND MURPHY, S. D.: Distribution of trimethyltin in various tissues of the male mouse. Toxicol. Lett. **17:** 43–48, 1983.
31. DYER, R. S.: Physiological methods for assessment of trimethyltin exposure. Neurobehav. Toxicol. Teratol. **4:** 659–664, 1982.
32. DYER, R. S., DESHIELDS, T. L., AND WONDERLIN, W. F.: Trimethyltin-induced changes in gross morphology of the hippocampus. Neurobehav. Toxicol. Teratol. **4:** 141–147, 1982.
33. DYER, R. S., HOWELL, W. E., AND WONDERLIN, W. F.: Visual system dysfunction following acute trimethyltin exposure in rats. Neurobehav. Toxicol. Teratol. **4:** 191–195, 1982.
34. DYER, R. S., WALSH, T. J., WONDERLIN, W. F., AND BERCEGEAY, M.: The trimethyltin syndrome in rats. Neurobehav. Toxicol. Teratol. **4:** 127–133, 1982.
35. DYER, R. S., WONDERLIN, W. F., AND WALSH T. J.: Increased seizure susceptibility following trimethyltin administration in rats. Neurobehav. Toxicol. Teratol. **4:** 203–208, 1982.
36. EVANS, C. J., AND SMITH, P. J.: Organotin-based antifouling systems. J. Oil Colour Chem. **58:** 160–168, 1975.
37. FISHMAN, R. A., AND CHAN, P. H.: Metabolic basis of brain edema. Adv. Neurol. **28:** 207–215, 1980.
38. FORTEMPS, E., AMAND, G., BOMBOIR, A., LAUWERYS, R., AND LATERRE, E. C.: Trimethyltin poisoning. Report of two cases. Int. Arch. Occup. Environ. Health **41:** 1–6, 1978.
39. FOX, D. A.: Pharmacological and biochemical evaluation of triethyltin's anticonvulsant effects. Neurobehav. Toxicol. Teratol. **4:** 273–278, 1982.
40. GERREN, R. A., GROSWALD, D. E., AND LUTTGES, M. W.: Triethyltin toxicity as a model for degenerative disorders. Pharmacol. Biochem. Behav. **5:** 299–307, 1976.
41. GRAHAM, D. I., DE-JESUS, P. V., PLEASURE, D. E., AND GONATAS, N. K.: Triethyltin sulfate-induced neuropathy in rats. Electrophysiologic, morphologic, and biochemical studies. Arch. Neurol. **33:** 40–48, 1976.
42. HARRY, G. J., AND TILSON, H. A.: The effects of postpartum exposure to triethyltin on the neurobehavioral functioning of rats. Neurotoxicology **2:** 283–296, 1981.
43. HARRY, G. J., AND TILSON, H. A.: Postpartum exposure to triethyl tin produces long-term alterations in responsivness to apomorphine. Neurotoxicology **3:** 64–71, 1982.
44. HOWELL, W. E., WALSH, T. J., AND DYER, R. S.: Somatosensory dysfunction following acute trimethyltin exposure. Neurobehav. Toxicol. Teratol. **4:** 197–201, 1982.
45. HUDSON. P. B., SANGER, G., AND SPROUL, E. E.: Effective system of bactericidal conditioning for hospitals. J. Am. Med. Assoc. **169:** 1549–1556, 1959.

46. HULEBAK, K. L., AND ANNAU, Z.: Trimethyltin toxicity in grasshopper mice (*Onychomys torridus*). Soc. Neurosci. Abstr. **7:** 748, 1981.
47. HUNTER, R. C.: Organotin compounds and their use for insect and mite control. Environ. Health Perspec. **14:** 47–50, 1976.
48. IDEMUDIA, S. O., AND MCMILLAN, D.E.: Spontaneous motor activitiy in pigeons: effects of pentobarbitial and trimethyltin (TMT). Fed. Proc. **44:** 494, 1985.
49. IDEMUDIA, S. O., AND MCMILLAN, D. E.: Effects of chemicals on delayed matching behavior in pigeons. III. Effects of triethyltin. Neurotoxicology (in press), 1985.
50. IDEMUDIA, S. O., MCMILLAN, D. E., AND CHANG, L. W.: Behavioral effects of trimethyltin chloride (TMT) in pigeons. Pharmacologist **25:** 214, 1983.
51. IDEMUDIA, S. O., MCMILLAN, D. E., AND CHANG, L. W.: Behavioral effects of triethyltin sulfate (TET) in pigeons. Pharmacologist **26:** 230, 1984.
52. JOHNSON, C. T., DUNN, A., ROBINSON, C., WALSH, T. J., AND SWARTZWELDER, H. S.: Alterations in regulatory and locomotor behaviors following trimethyltin exposure in the rat: a time and dose analysis. Neurosci. Lett. **47:** 99–106, 1984.
53. KIRSCHNER, D. A., AND SAPERSTEIN, V. S.: Triethyl tin-induced myelin edema—an intermediate swelling state detected by X-ray diffraction. N. Neurocytol. **11:** 559–569, 1982.
54. LEANDER, J. D., AND GAU, G. A.: Flavor aversions rapidly produced by inorganic lead and triethyl tin. Neurotoxicology **1:** 635–642, 1980.
55. LEOW, A. C., ANDERSON, R. M., LITTLE, R. A., AND LEAVER, D. D.: A sequential study of changes in the brain and cerebrospinal fluid of the rat following triethyltin poisoning. Acta Neuropathol. (Berl.) **47:** 117–121, 1979.
56. LEOW, A. C., TOWNS, K. M., AND LEAVER, D. D.: Effect of organotin and hexachlorophene on brain adenosine cyclic 3′,5′-monophosphate metabolism. Chem.-Biol. Interact. **27:** 125–132, 1979.
57. LOCK, E. A.: Increase in cerebral fluids in rats after treatment with hexachlorphene or triethyltin. Biochem. Pharmacol. **25:** 1455–1458, 1976.
58. LOCK, E. A.: The action of triethyltin on the respiration of rat brain cortex slices. J. Neurochem. **26:** 887–892, 1976.
59. LOCK, E. A., AND ALDRIDGE, W. N.: The binding of triethyltin to rat brain myelin. J. Neurochem. **25:** 871–876, 1975.
60. MACPHAIL, R. C.: Studies on the flavor aversions induced by trialkyltin compounds. Neurobehav. Toxicol. Teratol. **4:** 225–230, 1982.
61. MAILMAN, R. B., KRIGMAN, M. R., FRYE, G. D., AND HANIN, I.: Effects of postnatal trimethyltin or triethyltin treatment on CNS catecholamine, GABA, and acetylcholine systems in the rat. J. Neurochem. **40:** 1423–1429, 1983.
62. MARSHALL, L. F., BRUCE, D. A., GRAHAM, D. I., AND LANGFITT, T. W.: Alterations in behavior, brain electrical activity, cerebral blood flow, and intracranial pressure produced by triethyl tin sulfate induced cerebral edema. Stroke **7:** 21–25, 1976.
63. MASTROPAOLO, J. P., DACANAY, R. J., LUNA, B. H., TUCK, D. L., AND RILEY, A. L.: Effects of trimethyltin chloride on differential-reinforcement-of-low-rate responding. Neurobehav. Toxicol. Teratol. **6:** 193–199, 1984.
64. MCGEE, P. N., STONER, H. B., AND BARNES, J. M.: The experimental production of edema of the central nervous system of the rat by triethyltin compounds. J. Pathol. Bacteriol. **73:** 107–124, 1957.
65. MCMILLAN, D. E., BROCCO, M., WENGER, G. R., AND CHANG, L. W.: Effects of trimethyl tin injections in pigeons. Toxicologist **2:** 86, 1982.
66. MCMILLAN, D. E., BROCCO, M., WENGER, G. R., AND CHANG, L. W.: Effects of alkyltins on the schedule-controlled behavior of the pigeon. Neurobehav. Toxicol. Teratol. (in press), 1986.
67. MCMILLAN, D. E., CHANG, L. W., IDEMUDIA, S. O., AND WENGER, G. R.: Effects of trimethyltin and triethyltin on lever pressing, water drinking, and running in an activity wheel: associated neuropathology, Neurobehav. Toxicol. Teratol. (in press), 1986.
68. MCMILLAN, D. E., AND LEANDER, J. D.: Effects of drugs on schedule-controlled behavior. *In* Behavioral Pharmacology, ed. by S. D. Glick and J. Goldfarb, pp. 85–139, C. V. Mosby Co., St. Louis, 1976.
69. MILLINGTON, W. R., AND BIERKAMPER, G. G.: Chronic triethyltin exposure reduces the resting membrane potential of rat soleus muscle. Neurobehav. Toxicol. Teratol. **4:** 255–257, 1982.
70. MOORE, K. E., AND BRODY, T. M.: The effect of triethyltin on tissue amines. J. Pharmacol. Exp. Ther. **132:** 6–12, 1961.
71. MUSHAK, P., KRIGMAN, M. R., AND MAILMAN, R. B.: Comparative organotin toxicity in the developing rat: somatic and morphological changes and relationship to accumulation of total tin. Neurobehav. Toxicol. Teratol. **4:** 209–215, 1982.
72. MYERS, R. D., SWARTZWELDER, H. S., AND DYER, R. S.: Acute treatment with trimethyltin alters alcohol self-selection. Psychopharmacology **78:** 19–22, 1982.
73. NAGARA, H., SUZUKI, K., TIFFANY, C. W., AND SUZUKI, K.: Resistance of quaking mouse CNS to triethyl tin edema. Brain Res. **221:** 441–445, 1981.
74. NAGARA, H., SUZUKI, K., TIFFANY, C. W., AND SUZUKI, K.: Triethyl tin dose not induce intramyelinic vacuoles in the CNS of the quaking mouse. Brain Res. **225:** 413–420, 1981.
75. NOLAND, E. A., TAYLOR, D. H., AND BULL, R. J.: Monomethyl- and trimethyl-tin compounds induce learning deficiences in young rats. Neurobehav. Toxicol. Teratol. **4:** 539–544, 1982.
76. PIVER, W. T.: Organotin compounds: industrial application and biological investigations. Environ. Health Perspec. **4:** 61–79, 1973.
77. RASTOGI, S. K., WENGER, G.R., MCMILLAN, D. E., AND CHANG, L. W.: Effects of triethyltin and its interactions with *d*-amphetamine and chlorpromazine on responding under a multiple schedule of food presentation in rats. Neurobehav. Toxicol. Teratol. **7:** 239–242, 1985.
78. REITER, L. W., HEAVNER, G. B., DEAN, K. F., AND RUPPERT, P. H.: Developmental and behavioral effects of early postnatal exposure to triethyltin in rats. Neurobehav. Toxicol. Teratol. **3:** 285–293, 1981.
79. REITER, L. W., KIDD, K., HEAVNER, G. B., AND RUPPERT, P. H.: Behavioral toxicity of acute and subacute exposure to triethyltin in rat. Neurotoxicology **2:** 97–112, 1981.
80. REUHL, K. R., SMALLRIDGE, E. A., CHANG, L.W., AND MACKENZIE, B. A.: Developmental effects of triemethyltin intoxication in the neonatal mouse. I. Light microscopic studies. Neurotoxicology **4:** 19–28, 1983.
81. ROBINSON, I. M.: Effects of some organotin compounds on tissue amine levels in rats. Food Cosmet. Toxicol. **71:** 37–42, 1969.
82. ROSS, W. D., EMMETT, E. A., STEINER, J., AND TUREEN, R.: Neurotoxic effects of occupational exposure to organotins. Am. J. Psychiatry **138:** 1092–1095, 1981.
83. RUPPERT, P. H., DEAN, K. F., AND REITER, L. W.: Developmental and behavioral toxicity following acute postnatal exposure of rat pups to trimethyltin. Neurobehav. Toxicol. Teratol. **5:** 421–429, 1983.
84. RUPPERT, P. H., WALSH, T. J., REITER, L. W., AND DYER, R. S.: Trimethyltin-induced hyperactivity time course and pattern. Neurobehav. Toxicol. Teratol. **4:** 135–139, 1982.
85. SCHEINBERG, L. C., TAYLOR, J. M., HERZOG, I., AND MANDELL, S.: Optic and peripheral nerve response to triethyltin intoxication in the rabbit: biochemical and ultrastructural studies. J. Neuropathol. Exp. Neurol. **25:** 202–213, 1966.
86. SHELTON, W. W., NEVIS, A. H., AND COLLINS, G. H.: Triethyltin toxicity and brain impedance. Acta Neuropathol. (Berl.) **58:** 95–100, 1982.
87. SMITH, P., AND SMITH, L.: Organotin compounds and applications. Chem. Br. **11:** 208–226, 1975.
88. SQUIBB, R. E., CARMICHAEL, N. G., AND TILSON, H. A.: Behavioral and neuromorphological effects of triethyl tin bromide in adult rats. Toxicol. Appl. Pharmacol. **55:** 188–197, 1980.
89. SUZUKI, K.: Some new observations in triethyl-tin intoxication of rats. Exp. Neurol. **31:** 207–213, 1971.
90. SWARTZWELDER, H.S., DYER, R. S., HOLAHAN, W., AND MYERS, R. D.: Activity changes in rats following acute trimethyltin exposure. Neurotoxicology **2:** 589–593, 1981.
91. SWARTZWELDER, H. S., HEPLER, J., HOLAHAN, W., KING, S. E., LEVERENZ, H. A., MILLER, P. A., AND MYERS, R. D.: Impaired maze performance in the rat caused by trimethyltin treatment: problem-solving deficits and perseveration. Neurobehav. Toxicol. Teratol. **4:** 169–176, 1982.
92. SWARTZWELDER, H. S., HOLAHAN, W., AND MYERS, R. D.:Antagonism by *d*-amphetamine of trimethyltin-induced hyperactivity evidence toward an animal model of hyperkinetic behavior. Neuropharmacology **22:** 1049–1054, 1983.
93. TAN, L. P., AND NG, M. L.: The toxic effects of trialkyltin compounds on nerve and muscle. J. Neurochem. **29:** 689–696, 1977.
94. VALDES, J. J., MACTUTUS, C. F., SANTOS-ANDERSON, R. M., DAWSON, R., JR., AND ANNAU, Z.: Selective neurochemical and histological lesions in rat hippocampus following chronic trimethyltin exposure. Neurobehav. Toxicol. Teratol. **5:** 357–361, 1983.
95. VAN DER KERK, G. J. M., AND LUIJTEN, J. G. A.: Organotin compounds. III. Biocidal properties of organotin compounds. J. Appl. Chem. **4:** 314–319, 1954.
96. WALSH, T. J., GALLAGHER, M., BOSTOCK, E., AND DYER, R. S.: Trimethyltin impairs retention of a passive avoidance task. Neurobehav. Toxicol. Teratol. **4:** 163–167, 1982.
97. WALSH, T. J., MCLAMB, R. L., AND TILSON, H. A.: Organometal-induced antinociception: a time- and dose-response comparison of triethyl and trimethyl lead and tin. Toxicol. Appl. Pharmacol. **73:** 295–299, 1984.
98. WALSH, T. J., MILLER, D. B., AND DYER, R. S.: Trimethyltin, a selective limbic system neurotoxicant, impairs radial-arm maze performance. Neurobehav. Toxicol. Teratol. **4:** 177–183, 1982.
99. WATANABE, J.: Organotins. *In* Experimental Clinical Neurotoxicology, ed. by P. S. Spencer and H. H. Schaumburg, pp. 545–547, Williams & Wilkins, Baltimore, 1980.
100. WENGER, G. R., AND MCMILLAN, D. E.: Effects of triethyltin·SO_4 (TET) in mice responding under a multiple schedule of food presentation. Toxicologist **5:** 24, 1985.
101. WENGER, G. R., MCMILLAN, D. E., AND CHANG, L. W.: Behavioral effects of trimethyltin in two strains of mice. I. Spontaneous motor activity. Toxicol. Appl. Pharmacol. **73:** 78–88, 1984.
102. WENGER, G. R., MCMILLAN, D. E., AND CHANG, L. W.: Behavioral effects of trimethyltin in two strains of mice. II. Multiple fixed ratio, fixed interval. Toxicol. Appl. Pharmacol. **73:** 89–96, 1984.
103. WENGER, G. R., MCMILLAN, D. E., CHANG, L. W., ZITAGLIO, T., AND HARDWICK, W. C.: The effects of triethyltin and trimethyltin in rats responding under a DRL schedule of reinforcement. Toxicol. Appl. Pharmacol. **78:** 248–258, 1985.
104. WHITE, T. P.: Veber die Wirkungen des Zinns auf den thierischen organismus. Arch. Exp. Pathol. Pharmacol. **13:** 53–69, 1880.